THE PRIMARY STRUCTURE OF $tRNA_2^{Val}$ FROM *BACILLUS STEAROTHERMOPHILUS*

C. TAKADA-GUERRIER, H. GROSJEAN, G. DIRHEIMER* and G. KEITH*

*Laboratoire de Chimie Biologique, Université de Bruxelles, Rhode St-Genese, Belgique, and *Institut de Biologie Moléculaire et Cellulaire du C.N.R.S., rue Descartes, Strasbourg, France*

Received 25 November 1975

1. Introduction

In a previous paper we have reported the primary structure of $tRNA^{Phe}$ from *Bacillus stearothermophilus* (strain NCA 1518) [1]. This tRNA is aminoacylated by yeast phenylalanine: tRNA ligase [2] and has the composite sequence which seems to be involved in the aminoacylation process catalysed by this enzyme [3,4]. Phenylalanine: tRNA ligase from yeast also aminoacylates the $tRNAs^{Val}$ from *Bacillus stearothermophilus* in the presence of dimethylsulfoxide [2]. We purified one of these $tRNAs^{Val}$ and studied its primary structure in order to check whether or not it contained the same composite sequence. *B. stearothermophilus* $tRNA^{Val}$ is also very well aminoacylated under standard conditions by valine: tRNA ligase from yeast and *E. coli* [5]. Therefore it was also interesting to compare its structure with those of yeast and *E. coli* $tRNAs^{Val}$.

2. Materials and methods

B. stearothermophilus $tRNA^{Val}_2$ accounts for about 40% of the total acceptor activity for valine; it could be purified using chromatographic procedures similar to those used for the purification of *B. stearothermophilus* $tRNA^{Phe}$. Starting with the same bulk [^{32}P]tRNA [1], the first step was carried out on a BD-cellulose column, as was the case for $tRNA^{Phe}$ but $tRNA^{Val}$ was eluted using 1 M NaCl while $tRNA^{Phe}$ was only obtained by elution in the presence of ethanol.

The enriched $tRNA^{Val}$ fraction was further purified using three other column chromatographic procedures: (i) affinity column on Biogel P200 Hy-$tRNA^{Asp}$ (yeast $tRNA^{Asp}$ chemically coupled by the periodate oxidized 3′ end to hydrazine treated Bio-Gel P-200). This resin led to an important purification of $tRNA^{Val}_2$ having an anticodon G-A-C, complementary to the anticodon G-U-C of the resin bound tRNA species [6,7]. (ii) RPC-2 column [8] giving two peaks, one containing $tRNA^{Val}_1$ and the other $tRNA^{Val}_2$. (iii) Phenoxyacetylation on the $tRNA^{Val}_2$ containing peak followed by a BD-cellulose column according to Gillam et al. [9]. Further details concerning this purification will be published elsewhere [10].

The methods used for the determination of the nucleotide sequence were as previously described [11–13].

3. Results

Fig.1 shows a summary of overlapping fragments and derivatization of the primary structure of $tRNA^{Val}_2$. Its nucleotide sequence can be arranged in the familiar cloverleaf model of secondary structure (fig.2). Resemblances between this tRNA and the corresponding $tRNAs^{Val}$ from prokaryotes (*E. coli*) [14,15] and eukaryotes [16–20] and the $tRNAs^{Phe}$ from *Bacillus stearothermophilus* [1] and eukaryotes [21–23] are shown in fig.2A, 2B, 2C and 2D respectively.

$tRNA^{Val}_2$ contains 76 nucleosides including only 5 minor ones: hU, m^6A, m^7G, T and ψ, whereas *E. coli* [14,15], *Torulopsis utilis* [19], yeast [16–18] and mouse myeloma [20] $tRNAs^{Val}$ contain respectively 7–9, 12, 13 and 15 minor nucleosides.

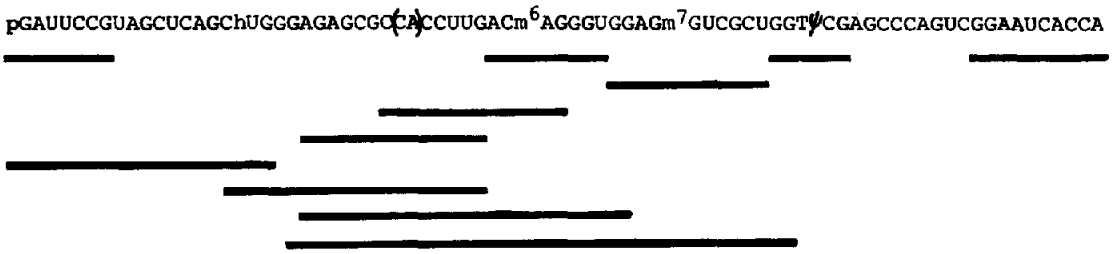

Fig.1. Primary structure of *B. stearothermophilus* $tRNA^{Val}_{2}$. Summary of overlapping fragments from exhaustive T_1 and pancreatic RNase digestions and partial T_1 RNase digestion. The sequence of the dinucleotide between brackets in position 28 and 29 from the 5'-end has not yet been established. Despite the absence of overlapping fragments between nucleotides 57 and 67 from the 5' end we could deduce this sequence by analysing the oligonucleotides obtained by complete T_1 and pancreatic RNase digestions.

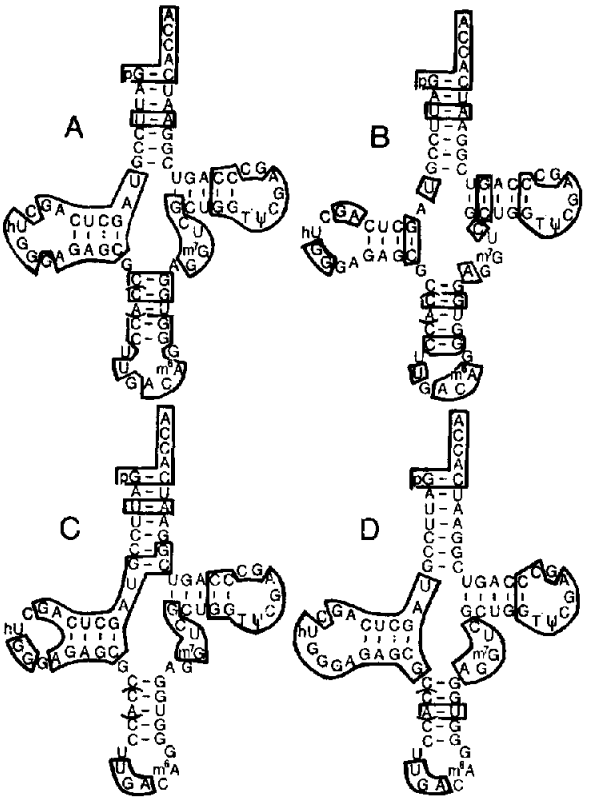

Fig.2. Comparison of the cloverleaf model of *B. stearothermophilus* $tRNAs^{Val}_{2}$ to other $tRNAs^{Val}$ or $tRNAs^{Phe}$ of known primary structure. In the boxes (A) common sequences with *E. coli* $tRNAs^{Val}$ (species 1, 2A and 2B) [14,15]; (B) common sequences with eukaryotic $tRNAs^{Val}$ (species 1 and 2a from bakers' yeast, $tRNA^{Val}$ from Torula yeast and from myeloma) [16–20]; (C) common sequences with *B. stearothermophilus* $tRNA^{Phe}$ [1]; (D) common sequences with eukaryotic $tRNAs^{Phe}$ (yeast, wheat germ, rabbit liver, calf liver [21–23].

$tRNA^{Val}_{2}$ contains m^6A at the 3'end of the anticodon as was found before in $tRNA^{Val}_{1}$ from *E. coli* [14] but not in *E. coli* $tRNA^{Val}_{2A \text{ or } 2B}$ which have a non-modified adenosine in this position.

Its hU loop is only 8 nucleotides long as in $tRNA^{Val}_{1}$ from *E. coli*, whereas other known $tRNAs^{Val}$ contain between 9 and 11 nucleotides.

The anticodon G-A-C is identical to that of K12 *E. coli* $tRNA^{Val}_{2A \text{ and } 2B}$ [15]. The extra loop contains 5 nucleotides as in most known $tRNAs^{Val}$, with the exception of torula yeast $tRNA^{Val}$ which has a smaller one (3 nucleotides only) [19].

B. stearothermophilus $tRNA^{Val}_{2}$ is very similar to either *E. coli* $tRNA^{Val}_{2A}$ (84% homology) or *E. coli* $tRNA^{Val}_{2B}$ (79% homology). The degree of homology is still high with *E. coli* $tRNA^{Val}_{1}$ (72%) whereas it is lower with eukaryotic $tRNAs^{Val}$: 60% with bakers' and torula yeast and 64% with mouse myeloma.

A detailed analysis of these sequences indicates that a clear-cut differentiation has occurred between $tRNAs^{Val}$ from prokaryotic and eukaryotic origins; a marked difference in sequence being observed in the stem of the dihydrouridine loop.

Despite these differences, *B. stearothermophilus* $tRNA^{Val}$ is fully charged by *E. coli* and yeast valine: tRNA ligases; conversely, valine: tRNA ligase from *B. stearothermophilus* fully charges both *E. coli* and yeast $tRNA^{Val}$, although at different rates [5]. This would indicate that specific nucleotides in the dihydrouridine stem have no essential discriminatory role for the recognition of the $tRNA^{Val}$ by the valine: tRNA ligase.

Homologies with $tRNAs^{Phe}$ from *B. stearothermophilus* (56%) from other prokaryotes (*Mycoplasma sp.*

58%) and *E. coli* (52%) [24,25] and from eukaryotes (mammals and wheat germ 62% and yeast 61%) [21–23] are even of the same extent as for eukaryotic tRNAs^{Val}. This probably explains why *B. stearothermophilus* tRNA$^{Val}_2$ can be misacylated by phenylalanine: tRNA ligase from yeast [2] since it contains the composite sequence involved in the aminoacylation process of phenylalanine tRNA ligase from yeast as suggested by Dudock et al. [3] and Kern et al. [4].

On the other hand it was demonstrated that valine: tRNA ligases from *B. stearothermophilus* and *E. coli* catalyses the aminoacylation of tRNA^{Phe} from yeast, whereas valine tRNA ligase from yeast charges both *E. coli* and *B. stearothermophilus* tRNA^{Phe} [2,5]. Other tRNAs from *E. coli* or yeast like tRNA^{Ile} and tRNA^{Met} are also aminoacylated in the presence of these ligases [5].

The existence of such cross-reactions between tRNAs and aminoacyl-tRNA ligases specific for phenylalanine and valine as well as important sequence similarities between all known tRNA^{Phe} and tRNA^{Val} suggest that all these tRNAs might derive from a common ancestor molecule [26–29].

Acknowledgements

We thank the EMBO for the award of a short term fellowship to one of us (C.T-G). This work was supported by grants from the Commissariat à l'Energie Atomique (France), from the Institut National de la Santé et de la Recherche Médicale (Contrat CRL n° 7310061), from the Fondation pour la Recherche Médicale Française and from the Action concertée de Biologie moléculaire - Etat Belge - ULB n° 099 - 72 - 1BIAB. C.T-G was a fellow of the Administration Générale de la Coopération au développement.

References

[1] Takada-Guerrier, C., Dirheimer, G., Grosjean, H. and Keith, G. (1975) FEBS Lett. 60, 286–289.
[2] Grosjean, H., Giegé, R., de Henau, S. and Narvaez, G. unpublished results.
[3] Dudock, B. S., di Peri, C., Scileppi, K. and Reszelbach, R. (1971) Proc. Natl. Acad. Sci. USA 68, 681.
[4] Kern, D., Giegé, R. and Ebel, J. P. (1972) Eur. J. Biochem. 31, 148.
[5] Giegé, R., Kern, D., Ebel, J. P., Grosjean, H., de Henau, S. and Chantrenne, H. (1974) Eur. J. Biochem. 45, 351.
[6] Eisinger, J. (1971) Biochem. Biophys. Res. Commun. 43, 854.
[7] Grosjean, H., Takada, C. and Petre, J. (1973) Biochem. Biophys. Res. Commun. 53, 882.
[8] Weiss, J. F. and Kelmers, A. D. (1967) Biochemistry 6, 2507.
[9] Gillam, I., Blew, D., Warrington, R. C., von Tigerstrom, M. and Tener, G. M. (1968) Biochemistry 7, 3459.
[10] Takada-Guerrier, C., Grosjean, H., Dirheimer, G. and Keith, G., in preparation.
[11] Sanger, F., Brownlee, G. G. and Barrell, B. G. (1965) J. Mol. Biol. 13, 373.
[12] Keith, G., Roy, A., Ebel, J. P. and Dirheimer, G. (1972) Biochimie 54, 1405.
[13] Keith, G. and Gilham, P. T. (1974) Biochemistry 13, 3601.
[14] Yaniv, M. and Barrell, B. G. (1969) Nature 222, 278.
[15] Yaniv, M. and Barrell, B. G. (1971) Nature 233, 113.
[16] Baev, A. A., Venkstern, T. V., Mirzabekov, A. D., Krutilina, A. I., Li, L. and Aksel'rod, V. D. (1967) Mol. Biol. (russ.) 1, 754.
[17] Bonnet, J., Ebel, J. P. and Dirheimer, G. (1971) FEBS Lett. 15, 286.
[18] Aksel'rod, V. D., Kryukov, V. M., Isaenko, S. N. and Baev, A. A. (1974) FEBS Lett. 45, 333.
[19] Takemura, S., Mizutani, T., Miyazaki, M. (1968) Jap. J. Biochem 63, 277.
[20] Piper, P. W. and Clark, B. F. C. (1974) FEBS Lett. 47, 56.
[21] Keith, G., Ebel, J. P. and Dirheimer, G. (1974) FEBS Lett. 48, 50.
[22] Dudock, B. S., Katz, G., Taylor, E. K. and Holley, R. W. (1969) Proc. Natl. Acad. Sci. US 62, 941.
[23] RajBhandary, U. L., Chang, S. H., Stuart, A., Faulkner, R. D., Hoskinson, R. M. and Khorana, H. G. (1967) Proc. Natl. Acad. Sci. US 57, 751.
[24] Barrell, B. G. and Sanger, F. (1969) FEBS Lett. 3, 275.
[25] Kimball, M. E., Szeto, K. S. and Söll, D. (1974) Nucleic Acids Research 1, 1721.
[26] Roe, B., Sirover, M. and Dudock, B. (1973) Biochemistry 12, 4146.
[27] Holmquist, R., Jukes, T. H. and Panghurn, S. (1973) J. Mol. Biol. 78, 91.
[28] Jukes, T. H. and Holmquist, R. (1972) Biochem. Biophys. Res. Commun. 49, 212.
[29] Takada-Guerrier, C. and Grosjean, H., in preparation.